Sapporo Medical Journal
Volume 59, Issue 01 January 2025

The Role of Tempol (4 hydroxy-tempo), a superoxide dismutase (SOD) mimetic, in Spinal Cord Injury Treatment

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Abstract— SCI includes both primary and secondary injuries, the former of which usually refers to a mechanical lesion to the spinal cord and the latter of which is caused by cellular and biological reactions to the main injury, including the vascular, neurological, and immunological systems.Oxidative stress is associated with SCI and can cause damage to cells. Repair enzymes such as SOD and quinine reductase play a crucial role in the repair and rebuilding process. Future studies on the possibility of tempol in the treatment of SCI hold a lot of promise.

Keywords: Spinal cord injury, Tempol, Superoxide dismutase, Neuroprotection

Background

Spinal Cord is a vital component of the central nervous system, serving as the primary pathway for communication between the rest of the body and the brain. Spinal Cord Injury (SCI) can cause damage to nerve roots or myelinated fiber tracts, often the result of accidents or traumatic events. (1) SCI can result in devastating consequences such as impaired mobility, pain, and autonomic dysfunction. (2) SCI triggers a complex series of cellular and molecular processes involving immediate and delayed injury mechanisms.(3) Oxidative stress, a key aspect of the secondary injury cascade, has been the focus of extensive research in recent years due to its critical role in the ongoing tissue damage and dysfunction following the initial traumatic event.
The inflammation aspect of secondary SCI is often the focus of neuroprotective agents due to its relatively good understanding. Primary and secondary SCI both boost the extracellular and intracellular mechanisms that hinder CNS axon regeneration.
(4) Reactive oxygen species (ROS), including the superoxide radical anion, hydroxyl radical, and hydrogen peroxide, are produced during metabolic and physiological processes. The effects of ROS are controlled by external antioxidants, as well as internal antioxidants including scavenger enzymes (SOD and GSH peroxidase). Oxidative stress is associated with SCI and can cause damage to cells. Repair enzymes such as SOD and quinine reductase play a crucial role in the repair and rebuilding process. Tempol, categorized as a nitroxide or a free radical undergoing one or two reduction cycles, may present a new approach to treating injuries that lead to the programmed death of neurons and glial cells.(5) The intricate nature of SCI, encompassing its pathological mechanisms and the formidable hurdles associated with neural regeneration, has been a persistent and formidable challenge for researchers.

This review emphasizes tempol's capacity to suppress polyol pathway activity, which preserves cellular glutathione and mitigates axonal degradation, indicating that tempol may serve as a possible treatment approach for spinal cord injury (SCI).

Pathophysiology of Spinal Cord Injury

The pathophysiology of spinal cord injury (SCI) encompasses primary and secondary injuries; the former typically refers to a mechanical injury to the spinal cord, while the latter results from cellular and biological responses to the primary injury, involving the immune, nervous, and vascular systems. This includes hemorrhage, ischemia, oxidative stress, inflammatory responses, neural cell death, demyelination, and scar formation, among others.(6)

Secondary spinal cord injury is delayed and progressive tissue damage that occurs subsequent to the main spinal cord injury. The blood-spinal cord barrier is disrupted during this secondary damage cascade, allowing inflammatory cells such neutrophils, T-cells, macrophages, and microglia to penetrate the injury site. (7) After blood vessel disruption results in hemorrhage in the spinal tissues, monocytes, neutrophils, T and B lymphocyte cells, and macrophages invade the affected areas. The production of inflammatory cytokines, including interleukin (IL)-1a, IL-1b, IL-6, and tumor necrosis factor (TNF)-α, occurs 6–12 hours after damage and is linked to this event. Neurons become inflamed when immune cells and inflammatory cytokines invade the body.

One of the most important therapeutic targets for stopping the progression of injuries is this secondary injury stage. There are other categories within the secondary stage, including acute, subacute, intermediate, and chronic stages. The subacute stage of SCI is characterized by neuroinflammatory responses, mitochondrial phosphorylation, and NOS production; the chronic stage is characterized by apoptosis and necrosis, axon degeneration, axon remodeling, glial scar formation, and spinal ischemia, vasogenic edema, and glutamate excitotoxicity.(Fig 1)(8)

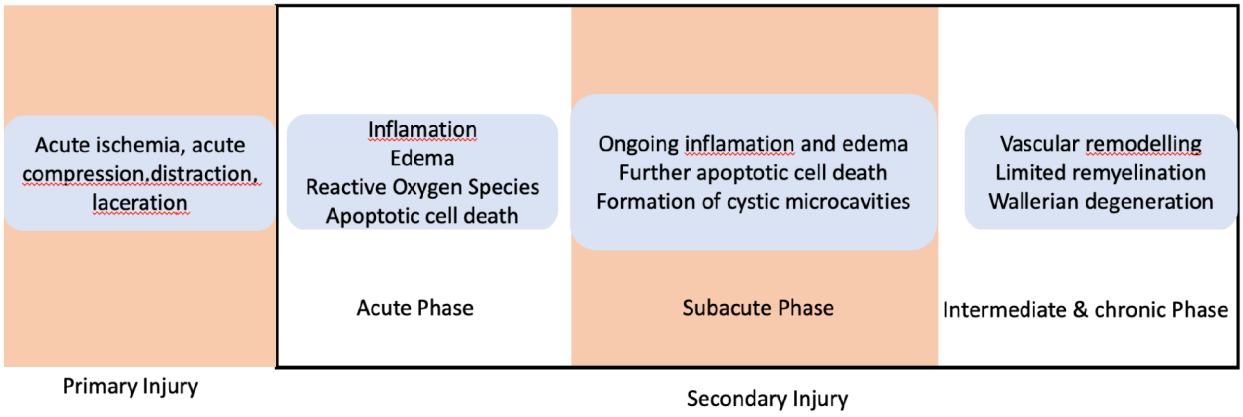

Fig 1.Pathophysiology of spinal cord injury

The disruption of the microvascular network leads to the infiltration of leukocytes and erythrocytes into the spinal cord tissue. (8) The accumulation of these immune cells within the injured site exerts compressive forces on the surrounding spinal tissues, further compromising the local blood perfusion and resulting in vasospasm. (9) This ischemic microenvironment,

characterized by decreased oxygen supply and impaired blood flow, provides a fertile ground for the overproduction of reactive oxygen species and reactive nitrogen species, which are major contributors to the oxidative stress that perpetuates the secondary injury cascade. The excess of these free radical species, including superoxide anions and hydrogen peroxide, can directly damage critical cellular macromolecules, such as lipids, proteins, and nucleic acids, impairing cellular function and ultimately leading to cell death. Apoptosis of oligodendrocytes initiates chronic demyelination, leading to anterograde neurodegeneration and the formation of fibers with disrupted myelin sheaths, a process known as Wallerian degeneration. Acute axonal degeneration (AAD) is another critical clinical manifestation observed in the early stages of spinal cord injury. This process involves the activation of cysteine protease calpain and further propagates axonal degeneration. AAD is triggered by a significant influx of calcium (Ca2+) into axons, and increased Ca2+ deposition heightens the risk of AAD. Wallerian degeneration is characterized by the development of retraction bulbs, a microtubular network that inhibits axonal regeneration. This anterograde degenerative mechanism is referred to as Wallerian degeneration.(10)

Demyelination, the destruction of myelin sheaths that insulate nerve fibers, occurs following spinal cord injury. This process impairs signal transmission along axons and leads to degeneration of both axons and oligodendrocytes, the myelin-producing cells.(9) During the secondary injury phase, oligodendrocytes undergo both necrotic and apoptotic cell death. Elevated glutamate levels trigger excessive calcium influx, which in turn provokes further cell death.(10) Oligodendrocyte damage is also driven by oxidative and nitrosative stress, depletion of the antioxidant glutathione, increased intracellular iron, and peroxisome dysfunction. (11) Reactive oxygen species produced by infiltrating neutrophils and activated microglia induce the release of pro-inflammatory cytokines such as TNFα, IL-2, and interferon γ, as well as proteolytic enzymes, further exacerbating oligodendrocyte apoptosis. (12)

Spinal cord injury leads to motor and sensory dysfunctions due to the cascade of primary and secondary damaging events. Many previous treatment strategies have not been successful in addressing SCI, as the pathological processes involved are complex. However, available therapeutic approaches can be broadly classified into neuroprotective, neuroregenerative, and immune-modulating pathways.(6)

Neuroprotection aims to protect neuronal structure and function, preserving neuronal integrity and decreasing neuron loss over time. This can delay disease progression and prevent further neurodegeneration. Pharmacological neuroprotective approaches can be divided into various subgroups, such as neurotransmitter agonists and antagonists, channel blockers, antioxidant agents, anti-apoptotic agents, and herbal/natural compounds, each targeting specific aspects of the degenerative cascade.(13)

The mechanisms by which reactive oxygen species and reactive nitrogen species are produced lead to an increased demand for ascorbic acid and alter the functionality of key antioxidant enzymes like superoxide dismutase, catalase, and glutathione. (14) Diminished activities of these critical antioxidant enzymes have been consistently observed in the context of neurodegenerative

disorders, highlighting the pivotal role that reduced antioxidant capacity plays in the pathogenesis of spinal cord injury. Antioxidants are chemical compounds that mitigate oxidative stress by preventing the oxidation of various molecules. As such, inhibitors of reactive oxygen species and reactive nitrogen species can counteract the oxidation of diverse bioactive molecules that occurs during the secondary phase of spinal cord injury. A wide range of molecules are utilized to control ROS and RNS generation. Antioxidant therapies can be categorized into two groups: those that inhibit the production of ROS and RNS, and those that prevent lipid peroxidation. (6)

Tempol role in Spinal Cord Injury

Tempol, a synthetic compound that mimics the catalytic activity of the superoxide dismutase enzymes, has been found to confer neuroprotective benefits in experimental models of spinal cord injury by attenuating oxidative stress. It is a membrane-permeable nitroxide compound, could allow increased Nrf2-dependent transcription, demonstrated efficacy as an antioxidant that effectively scavenges reactive oxygen species, promoting γGCL expression and resulting in greater glutathione synthesis. Consistent with this hypothesis, tempol has been shown to decrease NF-κB activation and increase Nrf2 activation. (1)(15) Tempol treatment also reduced expression of AKR1B10 in the degenerating axons. Inhibition of AKR1B10, an enzyme involved in the oxidation of lipid intermediates during lipid peroxidation, may be a key mechanism underlying tempol's protective effects against oxidative injury following SCI.(1) This protective function has been observed in multiple animal models examining brain ischemia, traumatic injuries, and mitochondrial dysfunction in the spinal cord. Tempol has been shown to exert a range of beneficial biological effects, including protective actions against radiation exposure, metabolic disorders, and shock, as well as safeguarding the heart, kidneys, and central nervous system.(16)

Tempol, a commercially available compound, is a potent nitric oxide scavenger and superoxide dismutase mimetic, offering several advantages that make it an excellent candidate for providing cellular protection.(17) Furthermore, in a spinal cord injury model, pretreatment with tempol has been shown to inhibit the expression of cyclooxygenase-2 and inducible nitric oxide synthase (iNOS), thereby decreasing NO production and reducing the injured area.(18)

SCI is characterized by the depletion of glutathione and greatly increased rates of lipid peroxidation, which are inhibited by tempol, a compound that mimics the catalytic activity of the human superoxide dismutase enzymes, selectively converting superoxide radicals into less reactive hydrogen peroxide and molecular oxygen.(19) In addition to tempol, other antioxidant therapies such as SOD mimetics, peroxynitrite decomposition catalysts, and compounds targeting specific oxidative pathways have demonstrated promising results in animal models of SCI, highlighting the potential therapeutic utility of targeting oxidative stress in the context of spinal cord injury. (14) Tempol had very similar effects on sparing of spinal cord tissue and locomotor recovery compared to sorbinil.(1) The effects of tempol were also non-additive with sorbinil when used in combination, consistent with involvement of similar mechanisms involving

the generation of ROS. Tempol could act by depleting superoxide generated by mitochondria or through NADPH oxidase, a consequence of polyol pathway activation, which otherwise would have been available for peroxynitrite formation.(Fig 2)

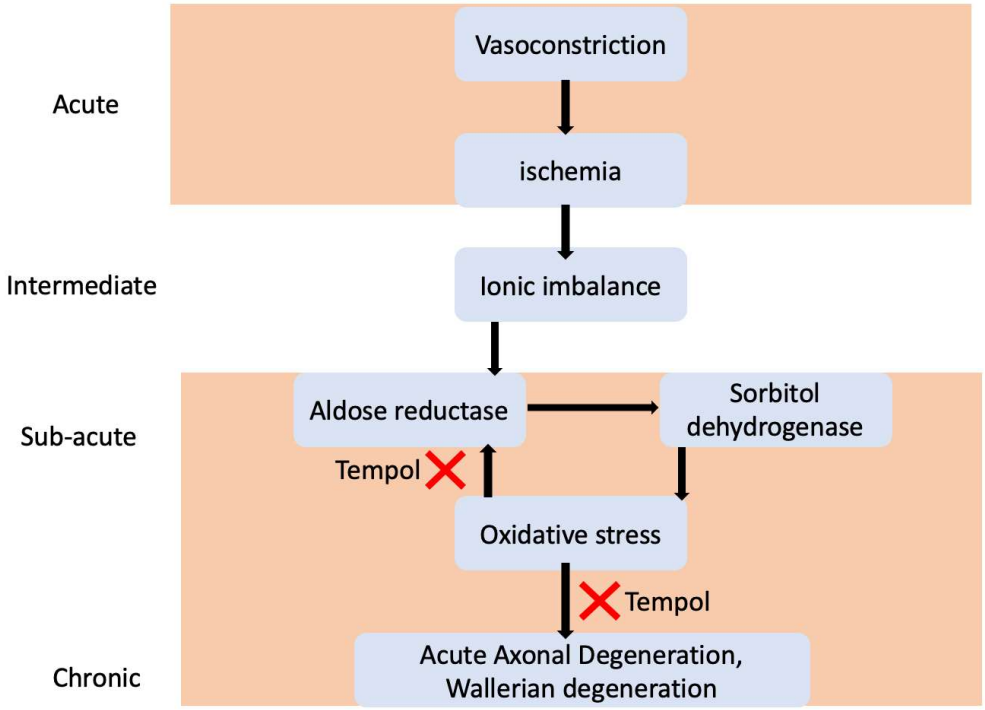

Fig.2 Pathophysiology and Role of Tempol in SCI

In traumatic injury, tempol therapy totally inhibited the amount of 3NT in mitochondria extracted from the injured brain at 12 hours after injury and in whole-tissue homogenates extracted from the injured cortical tissue at 1 hour after injury. Early temporal administration nearly entirely maintained mitochondrial respiration in addition to preventing mitochondrial oxidative damage.(20)

From another study about inflammatory pain, tempol, a SOD mimetic drug, decreased the expression of the NADPH oxidase subunit gp91phox mRNA in the spinal cord and paw skin, but peripheral injection of a superoxide anion donor increased it. Tempol also decreased the expression of TNFα, IL-1β, preproET-1, and COX-2 mRNA in the spinal cord and paw skin caused by superoxide anion. Actually, superoxide anion-induced pain and inflammation were lessened when tempol or other analgesics were used to target these mediators in the paw skin and spinal cord.(21)

Some in vivo studies found that tempol has shown a large benefit on SCI including effect on behavioural and functional recovery, reduction in inflammation, improved mitochondrial function, and neuroprotection. (Table 1)

Prior research indicates that tempol could represent a novel curative therapeutic approach for humans, rather than a preventive intervention. However, this strategy is typically not

recommended as a monotherapy, owing to the complex and multifaceted nature of SCI pathophysiology.

Table 1 In vivo studies of tempol

Dosage	Time	Sample	Results	
275mg/kg	6 weeks	Adult female Wistar mice	raised axonal area after injury in comparison to rats that were laminectomized but not injured (0 h), and $p < 0.00005$ substantial decreases in axonal area caused by tempol treatment as opposed to untreated or tempol + BSO treatment, and $p < 0.05$	(1)
100mg/kg	14 days	Sprague–Dawley male mice	After contusion SCI, the COX-2 mRNA/GAPDH ratio of the tempol-treated groups shows a similar trend, peaking at 8 hours and then declining, but the expression levels are much lower than those of the tempol-untreated groups.	(18)
3,10,30,100,300mg/kg	24 h	Young Adult Male CF-1 mice	At its peak 24 hours after injury, tempol (300 mg/kg) intraperitoneally at 15 minutes, 3, 6, 9, and 12 hours similarly reduced a-spectrin degradation by 45%. The same dosage schedule resulted in a notable, albeit small, reduction in hemisphere neurodegeneration at 7 days and enhanced 48-	(20)

			hour motor performance (17.4%, P<0.05).	
10,30,100mg/kg	5 h	male Balb/c mice	With statistical differences when compared to doses of 10 and 30 mg/kg, the dose of 100 mg/kg entirely reduced both mechanical hyperalgesia (3 h) and thermal hyperalgesia (1–5 h) as well as paw oedema between 1 and 5 h.	(21)

Conclusion

According to our research, which was based on reviews from many journals, tempol qualities can be used as a neuroprotective agent after spinal cord injury resulting in reduced apoptosis, enhanced motoneuron survival, and synapse maintenance.These findings show promise for tempol as an SCI treatment.

Abbreviations

SCI: Spinal cord injury;
SOD: Superoxide dismutase;
ROS: Reactive oxygen species;
RNS: Reactive nitrogen species;
AAD: Acute axonal degeneration;
iNOS:inducible nitric oxide synthase;
GSH:Glutathione; IL:Interleukin;
TNF:Tumor necrosis factor;
NADPH:Nicotinamide adenine dinucleotide phosphate;
NF-κB: Nuclear factor kappa B;
γGCL:γ-glutamate cysteine ligase;
Nrf2:Nuclear factor erythroid 2-related factor 2;
AKR1B10: Aldo-keto reductase 1B10;
COX-2:cyclooxygenase-2; mRNA:messenger ribonucleic acid;
preproET-1: preproendothelin-1.

Declarations

Ethics approval and consent to participate

Not required

Consent for publication
All authors have given their consent for publication.

Availability of data and material
Not applicable.

Competing interests
None declared

Funding
No funding sources

Authors' contributions
AP contributed to literature mining and manuscript writing. IDGD supervised the project. All authors read and approved the final manuscript.

Acknowledgements
Not applicable.

REFERENCES

[1] Zeman RJ, Brown AM, Wen X, Ouyang N, Etlinger JD. Tempol, a Superoxide Dismutase Mimetic, Inhibits Wallerian Degeneration Following Spinal Cord Injury by Preventing Glutathione Depletion and Aldose Reductase Activation. J Neurotrauma [Internet]. 2024 Sep 17 [cited 2024 Oct 2]; Available from: https://www.liebertpub.com/doi/10.1089/neu.2024.0137

[2] Zipser CM, Cragg JJ, Guest JD, Fehlings MG, Jutzeler CR, Anderson AJ, et al. Cell-based and stem-cell-based treatments for spinal cord injury: evidence from clinical trials. Lancet Neurol. 2022 Jul;21(7):659–70.

[3] Fatima G, Sharma VP, Das SK, Mahdi AA. Oxidative stress and antioxidative parameters in patients with spinal cord injury: implications in the pathogenesis of disease. Spinal Cord. 2015 Jan;53(1):3–6.

[4] Flack JA, Sharma KD, Xie JY. Delving into the recent advancements of spinal cord injury treatment: a review of recent progress. Neural Regeneration Res. 2022 Feb;17(2):283–91.

[5] Chiarotto GB, Drummond L, Cavarretto G, Bombeiro AL, de Oliveira ALR. Neuroprotective effect of tempol (4 hydroxy-tempo) on neuronal death induced by sciatic nerve transection in neonatal rats. Brain Res Bull. 2014 Jul;106:1–8.

[6] Hu X, Xu W, Ren Y, Wang Z, He X, Huang R, et al. Spinal cord injury: molecular mechanisms and therapeutic interventions. Signal Transduction and Targeted Therapy.

2023 Jun 26;8(1):1–28.

[7] Engaging pain fibers after a spinal cord injury fosters hemorrhage and expands the area of secondary injury. Exp Neurol. 2019 Jan 1;311:115–24.

[8] Anjum A, Yazid MD, FauziDaud M, Idris J, Ng AMH, Selvi Naicker A, et al. Spinal Cord Injury: Pathophysiology, Multimolecular Interactions, and Underlying Recovery Mechanisms. Int J Mol Sci. 2020 Oct 13;21(20):7533.

[9] Stewart AA, Bydon M, McGirt M, Bydon A. Spinal Cord: Anatomical Overview and Selected Pathologies. In: eLS. John Wiley & Sons, Ltd; 2009.

[10] Mataliotakis GI, Tsirikos AI. Spinal cord trauma: pathophysiology, classification of spinal cord injury syndromes, treatment principles and controversies. Orthopaedics and Trauma. 2016 Oct 1;30(5):440–9.

[11] Spaas J, van Veggel L, Schepers M, Tiane A, van Horssen J, Wilson DM 3rd, et al. Oxidative stress and impaired oligodendrocyte precursor cell differentiation in neurological disorders. Cell Mol Life Sci. 2021 May;78(10):4615–37.

[12] Fischer R, Maier O. Interrelation of oxidative stress and inflammation in neurodegenerative disease: role of TNF. Oxid Med Cell Longev. 2015 Mar 5;2015:610813.

[13] Lee KH, Cha M, Lee BH. Neuroprotective Effect of Antioxidants in the Brain. International Journal of Molecular Sciences. 2020 Sep 28;21(19):7152.

[14] Jomova K, Raptova R, Alomar SY, Alwasel SH, Nepovimova E, Kuca K, et al. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. Arch Toxicol. 2023 Oct;97(10):2499–574.

[15] Wilcox CS. Effects of tempol and redox-cycling nitroxides in models of oxidative stress. PharmacolTher. 2010 May;126(2):119–45.

[16] Kozlov AV, Javadov S, Sommer N. Cellular ROS and Antioxidants: Physiological and Pathological Role. Antioxidants. 2024 May 14;13(5):602.

[17] Amankwa CE, Kodati B, Donkor N, Acharya S. Therapeutic Potential of Antioxidants and Hybrid TEMPOL Derivatives in Ocular Neurodegenerative Diseases: A Glimpse into the Future. Biomedicines. 2023 Nov 1;11(11):2959.

[18] Quan HH, Kang KS, Sohn YK, Li M. Tempol reduces injury area in rat model of spinal cord contusion injury through suppression of iNOS and COX-2 expression. Neurol Sci. 2013 Sep;34(9):1621–8.

[19] Batinić-Haberle I, Rebouças JS, Spasojević I. Superoxide dismutase mimics: chemistry, pharmacology, and therapeutic potential. Antioxid Redox Signal. 2010 Sep 15;13(6):877–918.

[20] Deng-Bryant Y, Singh IN, Carrico KM, Hall ED. Neuroprotective Effects of Tempol, a Catalytic Scavenger of Peroxynitrite-Derived Free Radicals, in a Mouse Traumatic Brain Injury Model. Journal of Cerebral Blood Flow & Metabolism [Internet]. 2008 Mar 5 [cited 2024 Nov 10]; Available from: https://journals.sagepub.com/doi/10.1038/jcbfm.2008.10

[21] Bernardy CCF, Zarpelon AC, Pinho-Ribeiro FA, Calixto-Campos C, Carvalho TT, Fattori V, et al. Tempol, a Superoxide Dismutase Mimetic Agent, Inhibits Superoxide Anion-Induced Inflammatory Pain in Mice. BioMed Research International. 2017 Jan 1;2017(1):9584819.

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